IN THE CLAIMS

Claims 1-11 (Cancelled)

12. (amended) A method for preparing a protein with a C-terminal thioster, comprising:

(a) expressing a recombinant precursor protein in a host cell, the precursor protein comprising the protein fused to an intein and optionally a ~~binding~~ protein binding domain, the intein being selected from a naturally occurring intein, an intein derivative or an intein mutant, wherein the intein is capable of being ~~thiol induced cleavage~~ cleaved from the protein in the presence of 2-mercaptoethanesulfonic acid; and

(b) contacting the expressed precursor protein with ~~a thiol reagent~~ 2-mercaptoethanesulfonic acid and inducing cleavage of the intein from the precursor protein so as to form the target protein having the C-terminal thioester.

13. (amended) The method according to claim 12, wherein the intein is selected from Sce ~~Vma~~ VMA intein and Mxe ~~Gyr A~~ GyrA intein.

14. (amended) The method according to ~~of~~ claim 12, wherein the ~~thiol reagent is selected from 2-mercaptoethanosulfonic acid, thiophenol, dithiothreitol, and 3-mercaptopropionic acid~~ protein binding domain is a chitin binding domain.

15. (amended) The method according to claim 12, wherein the ~~precursor~~ protein is selected from a Bst DNA polymerase I large fragment, thioredoxin ~~and~~ or a cytotoxic protein.

16. (amended) The method according to claim 12, wherein the ~~precursor~~ protein is selected from a maltose binding protein and paramyosin.

17. (amended) A method for expressing a recombinant protein precursor, comprising:

inserting a nucleic acid sequence encoding a target protein into a plasmid at a multiple cloning site located upstream of and in frame with a fusion gene encoding an intein and a binding protein domain wherein ~~intein is selected from a naturally occurring intein, and intein derivative and an intein mutant modified intein; and~~

(i) the intein is selected from a naturally occurring intein, an intein derivative or an intein mutant; and

(ii) the multiple cloning site contains a linker and the linker sequence is selected from SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 or SEQ ID NO:4; and

introducing the plasmid into a host cell for expressing the recombinant precursor protein.

18. (previously amended) The method of claim 17, wherein the binding protein encoded by the nucleic acid is a chitin binding protein.

Claims 19-20 (cancelled)

21. (previously added) The method according to claim 17, wherein the plasmid is a pTXB plasmid.

22. (amended) A method of modifying a protein by ligating a synthetic peptide or synthetic second protein *in vitro* to the ~~to an inactive~~ protein ~~so as to restore protein activity~~, comprising:

(a) expressing in a host cell, the protein fused to one of an intein, an intein derivative or ~~an intein mutant intein~~ a mutant intein, wherein the intein is capable of thiol induced cleavage;

(b) inducing intein mediated cleavage of the protein by adding ~~a thiol reagent~~ 2-mercaptoethanesulfonic acid so as to form a C-terminal thioester on the protein;

(c) preparing a synthetic peptide or a synthetic second protein having an N-terminal cysteine; and

(d) ligating ~~the inactive form of~~ the protein to the synthetic peptide or a synthetic second protein to modify the protein ~~activity~~.

23. (prevously added) The method according to claim 22, wherein the protein prior to modification is a cytotoxic protein.

24. (amended) ~~the method of claim 21, wherein the cytotoxic protein is a restriction endonuclease~~ The method according to claim 22, wherein the intein, the intein derivative or the mutant intein in step (a) is optionally fused to a protein binding domain.

25. (amended) A method of labeling a target protein, comprising:

(a) expressing a recombinant precursor protein in a host cell, the precursor protein comprising the target protein fused to an intein

and optionally a binding protein domain, the intein being selected from a naturally occurring intein, an intein derivative or an intein mutant, wherein the intein is capable of thiol induced cleavage;

(b) cleaving the precursor protein in the presence of ~~a thiol reagent~~ 2-mercaptoethanosulfonic acid so as to form the target protein having a C-terminal thioester;

(c) preparing a synthetic peptide or protein having a marker and an N-terminal cysteine; and

(d) ligating the target protein with the synthetic peptide or protein for ~~labelling~~ labeling the target protein.

26. (previously added) The method according to claim ~~24~~ 25, wherein the marker is selected from the group consisting of a fluorescent marker, a spin label, an affinity tag, and a radiolabel.

27. (previously added) The method according to claim ~~24~~ 25, wherein the peptide fragment is an antigenic determinant.

28. (amended) A method for ligating a ~~first target protein with a second target protein, the method~~ synthetic protein or peptide to an inactive protein so as to restore protein activity, comprising

(a) expressing in a host cell, a fusion protein comprising the first target protein fused ~~to an intein having an N-terminal cleavage activity wherein the fusion protein is expressed from a first plasmid~~ at the C-terminus to one of an intein, an intein derivative or an intein mutant wherein the fusion protein is expressed from a plasmid;

(b) ~~contacting the fusion protein of step (a) with a thiol reagent for inducing cleavage of the intein to produce a C-terminal thioester on the first target protein; and~~ inducing intein mediated cleavage of the protein by adding 2-mercaptoethanesulfonic acid so as to form a C-terminal thioester on the protein;

(c) ~~combining a mixture for permitting ligation, the C-terminal thioester on the first target protein and a thioester reactive N-terminal amino acid on the second target protein~~ preparing a synthetic protein or peptide having an N-terminal cysteine; and

(d) ligating the inactive form of the protein to the synthetic peptide to restore protein activity.

29. (previously amended) The method according to claim 28, wherein the protein is a cytotoxic protein.

30. (previously amended) The method of claim 29, wherein the cytotoxic protein is a restriction endonuclease.

Claims 31-33 (Cancelled)